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The development and maintenance of neurons in the inner ear
Vývoj a funkce neuronů vnitřního ucha

Bachelor´s thesis

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Declaration

I declare that I carried out this bachelor thesis independently and only with the cited sources, literature, and other professional sources. I declare that this thesis has not been used to gain any other academic title.

Prague, 1.6.2020 Natália Wirthová

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Abstract

Hearing loss is among the most frequent disabilities. Neurosensory hearing loss is permanent and results from the death of neurons or sensory cells, which have little ability to regenerate in the inner ear (cochlea). Identifying the genes that are associated with generating differentiated and functional sensory cells, neurons, and with hearing loss could provide tools for neurosensory regeneration therapy and preventive measures. Recent data suggest that the prevention of neuronal loss and enhancement of long-term maintenance of neurons are the most important targets for the immediate future. This work is focused on transcription factors and signaling pathway networks that regulate the development and maintenance of neurons in the inner ear.

Key words: hair cell, neurons, inner ear, transcription factors, signaling pathways, hearing loss

Abstrakt

Ztráta sluchu patří mezi nejčastější postižení. Neurosenzorická ztráta sluchu je trvalá a je důsledkem smrti neuronů nebo senzorických buněk, které mají malou schopnost regenerace. Je zřejmé, že identifikace genů, které jsou spojeny s diferenciací funkčních senzorických buněk, neuronů a se ztrátou sluchu, by mohla ukázat nové možnosti terapie zaměřené na regeneracipoškozených neurosenzorických buněk.. Nejnovější údaje naznačují, že prevence ztráty neuronů a zlepšení dlouhodobého přežívání neuronů jsou nejdůležitějšími cíli pro nejbližší budoucnost. Tato práce je zaměřena na zmapování současných poznatků týkajících se signálních a regulačních drah transkripčních faktorů, které jsou nezbytné pro vývoj a funkci neuronů ve vnitřním uchu.

Klíčové slova: vláskové buňky, neurony, vnitřní ucho, transkripční faktory, signální dráhy, ztráta sluchu

List of Abbreviations

ASCL 1	Achaete-scute family transcription factor 1
ATOH1	Atonal homolog 1
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
BHLH	Basic helix-loop-helix
CADH23	Cadherin 23
CKO	Conditional knock-out
DLL1	Delta-like protein 1
E	Embryonic day
EYA1	Eyes absent homolog 1
GATA3	GATA-binding factor 3
HMG	High-mobility group
ISL1	Insulin gene enhancer protein 1
JAG2	Jagged 2
LHX3	LIM homeobox protein 3
LIM	LIN11-ISL1-MEC3
NEUROD1	Neurogenic differentiation factor 1
NEUROG1	Neurogenin 1
NOTCH1	Neurogenic locus notch homolog protein 1
NTF3	Neurotrophin 3
NTRK2	Neurotrophic Receptor Tyrosine Kinase 2
NTRK3	Neurotrophic Receptor Tyrosine Kinase 3
P	Postnatal day
PCDH15	Protocadherin 15
POU	PIT1-OCT1/2-UNC86
POU4F1	POU domain class 4 transcription factor 1
SGN	Spiral ganglion neuron
SIX1	Sine oculis homeobox homolog 1
SNHL	Sensorineural hearing loss
SOX2	Sex determining region Y-box factor 2
SR	Spontaneous firing rate
TBX1	Testis-specific T-box protein

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1. Introduction

Hearing impairment, partial or total inability to hear sounds, is among the top 10 disabilities of today's society. Around 6% of the world's population live with hearing loss; 34 millions of them are children. Third of the population over 65 is affected by disabling hearing loss. In 30 years, one in every ten people -over 900 million people- will have a hearing impairment.

Hearing loss can be conductive, sensorineural, or both (mixed loss). The causes of hearing loss are congenital or acquired. Congenital hearing loss is caused by a variety of genetic factors as well as by complications during pregnancy. Acquired hearing loss can appear at any time during life. The most common causes of acquired hearing loss include noise, aging, and diseases or infections (World Health Organization, 2020).

Sensorineural hearing loss (SNHL) is the far more common type of hearing loss. SNHL is caused by damage to cochlear sensory hair cells, neurons or neuronal fibers of the auditory pathway. The cells of sensory epithelia and auditory neurons have a low ability to regenerate. Thus, the damage of neurosensory cells is permanent. Current options to compensate for hearing loss include hearing aids and cochlear implants (Medical News Today, 2018).

According to the latest data, the prevention of neuronal loss may be a key to the regeneration of neurosensory epithelia within the inner ear. Recent studies focus on possibilities for neuronal replacement, including exogenous stem cell transplantation, endogenous cell source replacement, and induced expression of specific transcription factors. Therefore, understanding and identifying individual transcription factors and signal proteins involved in the development and survival of auditory neurons are crucial for future treatment of hearing loss (Shi and Edge, 2013). In this thesis, I reviewed key transcription factors and signaling pathways affecting the development, differentiation, and survival of the mammalian inner ear's neurons.

2. Morphology of the ear

The ear consists of three components: the outer ear, the middle ear, and the inner ear. The outer ear extends from the outside of the head to the eardrum. It is followed by the middle ear, which is an air-filled structure, medial to the eardrum. The inner ear represents a system of interlinked channels and chambers filled with fluids, medial to the middle ear (Figure 1).

2.1. Morphology of the outer ear

The outer ear contains three parts: the auricle, the external acoustic meatus, and the tympanic membrane or the eardrum. The auricle is a cartilaginous structure of the outer ear. It opens into the external acoustic meatus, the passage linking the auricle with the tympanic membrane. The tympanic membrane is composed of a thin layer of the connective tissue and epithelial layers. The purpose of the outer ear is to collect and amplify sound waves proceeding to the inner parts of the ear. Incoming sound causes the eardrum to vibrate as it transmits the wave further to the ear.

2.2. Morphology of the middle ear

The middle ear includes the area between the tympanic membrane and the oval window and the round window of the inner ear. The tympanic cavity within a temporal bone is filled with air and houses chain of three interlinked auditory ossicles: the malleus, the incus, and the stapes. The long handle of

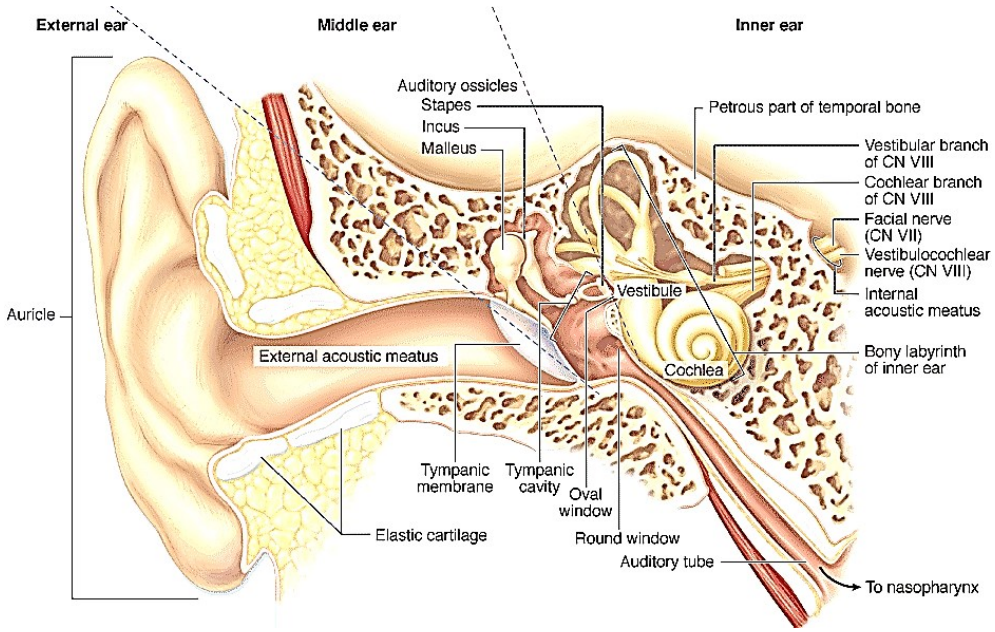

Figure 1. Morphology of the ear. The auricle, the external acoustic meatus, and the tympanic membrane form the outer ear. The middle ear consists of three ossicles: the malleus, the incus, and the stapes and is connected to the nasopharynx by the auditory tube. The middle ear is followed by the inner ear, which contains the cochlea and the vestibular system. Adapted from (Mescher, 2010).

the malleus is linked with the inner surface of the tympanic membrane, and the footplate of the stapes is associated with the oval window of the inner ear. Transmitted vibrations are amplified through the ossicles, resulting in 15-20 times higher sound pressure on the side of the oval window. The reason is a much smaller area of the oval window compared to the area of the tympanic membrane, along with the level-like composition of the auditory ossicles. The middle ear is connected to the sidewall of the nasopharynx through the Eustachian tube functioning as the pressure equalizer (Langmeier, 2009; Stevens and Warshofsky, 1965).

2.3. Morphology of the inner ear

The inner ear can be found in the petrous portion of the temporal bone. It consists of the connected sections of the bony labyrinth containing the cochlea, the vestibule, and three semi-circular canals. Inside the bony labyrinth remains a structure called the membranous labyrinth. The membranous labyrinth includes the cochlear duct, the saccule, the utricle, three semi-circular ducts, and associated ampullae (Figure 2). Spatial stability of membranous labyrinth is maintained by connection with the fibrous trabeculae, extending from layered tissue of squamous epithelial cells, which line the wall of the bony labyrinth (Konrádová, 2002).

Both parts of the inner ear are filled with fluids differing in the composition of ions. Perilymph, formed by filtration of the blood plasma, is located inside of the bony labyrinth. It is rich in sodium ions, poor in potassium ions, and akin to the extracellular fluid. Endolymph is produced by the epithelium of membranous cochlea called *stria vascularis* and fills the membranous labyrinth. Its composition is similar to intracellular fluid, containing a low concentration of sodium ions and a high concentration of potassium ions. Both perilymph and endolymph are continuously secreted and drained. Under physiological conditions, there is a balance between the two processes. Due to the differences in the composition of these fluids, the endocochlear potential can be detected within the cochlea. While being the highest transepithelial voltage in the body, it is considered to be the main driving force for sensory transduction in the hair cells. Besides the endocochlear potential, the intrastrial potential in the extracellular compartment of the *stria vascularis*, known as intrastrial space, is recorded. The intrastrial potential itself is an essential factor for the initiation of the endocochlear potential (Nin *et al.*, 2008; Sterkers, 1988).

2.3.1. The cochlea

The complex forming the cochlea contains the necessary equipment for hearing. The bony cochlea is a tubular structure approximately 35 mm long and 2.75 times coiled around a central axis called the modiolus. The tip of the cochlea is the apex, and the lower part forms the base. Within the bony cochlea, the spiral cochlear duct is situated and divides the cochlea into three individual sections: *scala vestibuli*, *scala media*, and *scala tympani*. The triangular *scala media* represents membranous cochlear duct containing endolymph, *scala vestibuli*, and *scala tympani* are filled with perilymph. *Scala vestibuli* leads

from the oval window of the inner ear to the tip of the cochlea known as the helicotrema, where it communicates with *scala tympani*, beginning at the round window of the middle ear. *Scala media* is bounded above by the vestibular membrane referred to as the Reissner´s membrane and bounded below by the basilar membrane, carrying the organ of Corti. The outer side is lined with thickened periost (Konrádová, 2002).

The organ of Corti is the sensory epithelium of the cochlea. It is formed by two rows of sensory hair cells, numerous supporting cells, and interconnecting nerve fibers. The groups of hair cells are separated by the inner tunnel, originating from a sector of the supporting cells. Dendrites of the bipolar neurons situated in the modiolus innervate sensory hair cells through synapses on the basal domain. The apical connection of hair cells and supporting cells creates the layer known as *lamina reticularis*. The tectonic membrane, located above *lamina reticularis*, is rich in glycoproteins produced by cells of *limbus spiralis*. Within the organ of Corti, the sound is transmitted along the nerve to the brain like an electric signal. In response to the perilymph movement in *scala tympani*, the basilar membrane bows upward, causing stereocilia of hair cells to hit the tectonic membrane. This movement initiates the depolarization of hair cells and generates an action potential (Králiček, 2004).

2.3.2. The vestibule

The vestibule includes two chambers: the utricle and the saccule. As a static part of the vestibular system, it responds to linear acceleration and the position of the head relative to gravity. The utricle and

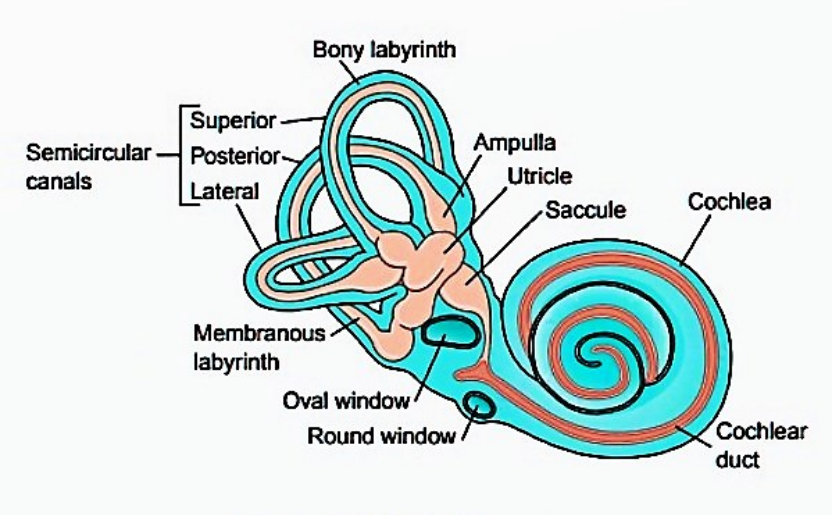

Figure 2. Morphology of the inner ear. The inner ear contains the hearing and balance apparatus. It consists of the bony and membraneous labyrinth. The bony labyrinth is divided into three main regions: the cochlea, the vestibule, and three semi-circular canals. The membraneous labyrinth contains the cochlear duct, the saccule, the utricle, three semi-circular ducts, and associated ampullae Adapted from (BrainKart.com, 2018-2020).

the saccule are called the otolithic organs. Both house sensory epithelium, known as the maculae. The macula in the saccule is orientated in the vertical plane, while the macula in the utricle is oriented horizontally. The maculae contain supporting cells and receptor hair cells, which are embedded in the gelatinous otolithic membrane. The surface of the layer is weighted by small structures known as the otoliths, particles composed of protein and calcium carbonate. In response to change in the position of the head, the otolithic membrane shifts in its location. The movement causes microvilli of hair cells to bend to generate an action potential. Associated neurons of the vestibular system carry resulting potential further to the brain (Ekdale, 2016).

2.3.3. The semi-circular canals

The system of semi-circular canals includes superior, horizontal, and posterior canals, placed at nearly right angles to one another. Within the canals, three semi-circular ducts are located, each of them being sensitive to the movement at different angles. As a kinetic part of the vestibular system, they respond to angular acceleration and rotation of the head. Each canal expands into an ampulla, the bulge with sensory epithelium forming the *crista ampularis*. The sensory epithelium consists of supporting cells and receptor hair cells, which are coated by the substance similar to the otolithic membrane, the cupula. Its structure and function are much like the macula´s, but the cupula contains no otoliths. During the initial rotation of the head, endolymph remains in its position due to the static interia. The delay causes displacement of stereocilia of the hair cells and generates an action potential. However, when moving at a constant velocity, the cupula remains in its position, stereocilia are not deflected, and the membrane potential of the cells normalizes. In such a case, no action potential is carried to the brain (Králiček, 2004; Konrádová, 2002).

3. Inner ear cells

The sensory receptors for hearing and balance are hair cells of inner ear sensory epithelia. Apart from sensory hair cells, the sensory epithelium consists of non-sensory supporting cells, which are necessary for the development, function, and maintenance of sensory hair cells. Hair cells are innervated by neurons. Within the inner ear, two distinguishable systems of neurons are located, known as the descending neuronal pathway and ascending neuronal pathway.

3.1. The sensory epithelium

The sensory epithelia of the inner ear consist of the auditory sensory epithelium and the vestibular sensory epithelium. The auditory sensory epithelium refers to the organ of Corti within the cochlear region. The vestibular sensory epithelium includes the maculae of the saccule and the utricle, and the *crista ampullaris* of the semi-circular canals within the vestibular system. Both of the sensory epithelia contain interconnected mechanosensory hair cells and non-sensory supporting cells (Schwander, 2010).

3.1.1. Sensory hair cells

The inner ear hair cells represent modified epithelial cells. They serve as highly specialized mechanoreceptors converting incoming sound waves into the electrochemical signals responsible for the sense of hearing and balance (Hudspeth, 2014). Hair cells are embedded in a layer of the neuroepithelium. Their structure involves the bundle of stereocilia and a single cilium called the kinocilium on the apical end, along with the afferent and efferent nerve fibers forming synapses on the basal end. Individual hair cells are separated by the supporting cells (Figure 3) (Hudspeth and Corey, 1977).

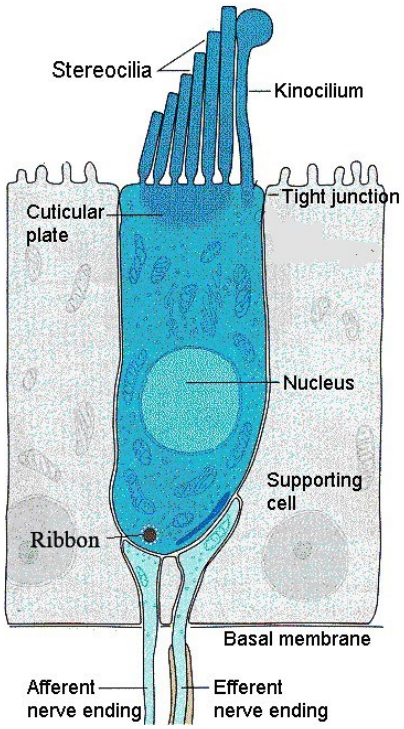

Figure 3. Structure of the hair cell. The bundle of stereocilia and a single true cilium are located on the apical side of sensory hair cell. Peripheral projections form synapses on the basal end. Each sensory hair cell is surrounded by non-sensory supporting cells (Kandel, 2000).

Stereocilia are derived microvilli projections extending from the apical surface of the hair cell. The bundle includes dozens of individual stereocilia organized in rows of decreasing height and arranged in a bilaterally symmetric fashion (Rzadzinska *et al.*, 2004). Stereocilia are supported by hundreds of actin-rich parallel filaments coated with diverse isoforms of myosin. Actin filaments are polarized. The minus ends proceed towards the base, while the plus ends point to the apical side, allowing stereocilia to grow by adding actin monomers to their tips (Tilney, 1980; Zhang *et al.*, 2012). The kinocilium is a single true cilium containing one central and nine surrounding pairs of microtubules. It is located in the hair bundle, typically next to the tallest stereocilia. In certain forms of the sensory epithelia, such as the mammalian cochlea, the kinocilium degenerates after the birth, even though the system of mature hair cells remains fully functional (Hudspeth and Jacobs, 1979). However, the presence of the kinocilium during early development stages is crucial since having a significant mechanosensitive role (Kindt, 2012).

Adjacent stereocilia are linked together by a variety of extracellular cross-links. Those include the side links connecting stereocilia along the shafts, termed ankle links at their bases (Fettiplace and Kim, 2014), and the “tip links.” The “tip links” are asymmetric structures consisting of CDH23 and PCDH15 complexes (Kazmierczak *et al.*, 2007) that connect the tips of shorter stereocilia to the body of subsequent taller stereocilia. The bending of stereocilia towards the tallest cilium causes the “tip links” to stretch and mechanically-sensitive transducer channels on the surface of stereocilia to open. Potassium and calcium ions flow inside and induce neurotransmitter release. Conversely, the tilting of

stereocilia away from the tallest cilium leads to the closure of the channels, hyperpolarization of hair cells, and inhibition of synaptic transmission (Hudspeth and Corey, 1977; Schwander, 2010).

The population of hair cells in the inner ear is heterogeneous, differing in both morphology and physiology. The auditory sensory epithelium consists of inner and outer hair cells. The flask-shaped inner hair cells form a single row, and the columnar outer hair cells are arranged in three rows. The convention of the mechanical stimuli into electrical signals within the cochlea is carried out by inner hair cells, while outer hair cells act as the "cochlea amplifier." The vestibular sensory epithelium carries Type I and Type II hair cells (Purves *et al.*, 2004). Unlike hair cells within the mammalian cochlea, the hair cells of the vestibular system are capable of a partial recovery of damaged cells (Taylor *et al.*, 2018).

3.1.2. Supporting cells

The supporting cells are essential for proper development and maintenance of sensory hair cells in the mammalian inner ear. Healthy supporting cells are vital for mechanical support and integrity of the epithelium, functioning as essential regulators of the ion transport as well as the removers of damaged tissue. Supporting cells ensure the survival of spiral ganglion neurons after hair cell loss, which makes their presence crucial for proper neural function. The characteristics described above make them determiners of the survival hair cell and neurons (Monzack and Cunningham, 2013; Zilberstein, 2012).

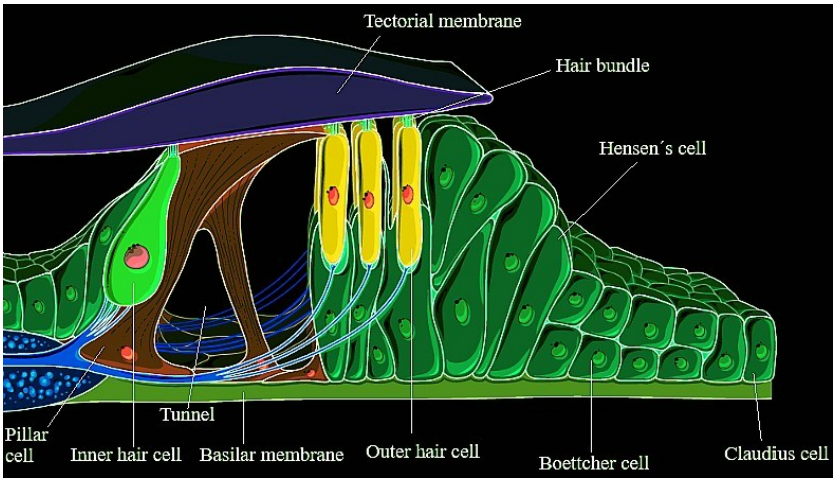

Figure 4. The organ of Corti. The organ of Corti is formed by two rows of sensory hair cells, numerous supporting cells, and interconnecting nerve fibers. Inner phalangeal cells and Dieters' cells surround the sensory hair cells, providing mechanical support. Pillar cells separate the outer hair cells and the inner hair cells while forming the tunnel of Corti. Claudius cells and Boettcher cells are not associated with sensory hair cells. Adapted from (Servier Medical Art, 2020).

The supporting cells contain highly developed cytoskeletal system and a large number of microtubules providing mechanical durability (Henderson *et al.*, 1994). Supporting cells are linked to each other and to hair cells by numerous intercellular connections. Within the cochlear region, supporting cells are connected using gap junctions, which consist of various isoforms of connexins. Systematic exchange of ions through the

junctions is required for the proper function of the auditory system. Functional defects lead to disruption of homeostasis and may cause hearing loss. Tight-adherens junctions connect supporting cells and hair cells. (Nunes *et al.*, 2006; Zhang *et al.*, 2005).

While the population of supporting cells within the vestibular system is considered to be homogenous, a number of different subtypes of supporting cells can be found in the mature organ of Corti (Figure 4). Such cells include inner phalangeal cells, pillar cells, Dieters' cells, Hensen's cells, Claudius cells, and Boettcher cells. Dieters' cells surround the outer hair cells, providing mechanical support. Pillar cells separate the outer hair cells and the inner hair cells while forming the tunnel of Corti. Claudius cells and Boettcher cells, unlike the other cells, are not associated with sensory hair cells (Monzack and Cunningham, 2013; White *et al.*, 2006).

Supporting cells in the cochlea have an indispensable role in the regeneration of the auditory sensory epithelium. Some subtypes of supporting cells show the capability of renewed proliferation and transdifferentiation into hair cell-like cells, which can create connections with auditory neurons (McGovern *et al.*, 2019; Shu *et al.*, 2019).

3.2. Neurons in the inner ear

Two distinguishable systems of neurons exist within the mammalian inner ear, known as the descending neuronal pathway and ascending neuronal pathway.

The pathway descending from the cortex is called the efferent system or the olivocochlear system. While being unique to the auditory region, it consists of efferent neurons subdivided into medial olivocochlear efferents and lateral olivocochlear efferents. The efferents are derived from facial branchial motor neurons. The groups differ in the neuron bodies' location and the degree of the myelination. Thicker myelinated medial efferents form synapses with outer hair cells. Thin unmyelinated lateral efferents innervate the dendrites of nerve fibers connecting inner hair cells (Elgoyhen *et al.*, 2019; Maison, 2003). The pathway uses acetylcholine as the major neurotransmitter (Elgoyhen *et al.*, 2001; Lustig *et al.*, 2001). The efferent system is involved in the improvement of signal detection, the functioning of outer hair cells (Elgoyhen *et al.*, 2019), and protection of the cochlea from acoustic damage. The difference in the olivocochlear system efficiency is an essential factor of the vulnerability to permanent acoustic injury (Maison and Liberman, 2000).

Neurons forming the ascending neuronal pathway innervate the sensory epithelia of the inner ear and transmit received information to the brain. The somata of neurons form two individual ganglia. The vestibular ganglion encircles the surface of the inner ear, whereas the auditory ganglion twists along the length of the cochlear duct. Peripheral neuronal processes innervate hair cells within the auditory sensory epithelium and the vestibular sensory epithelium. The processes are usually referred to as the dendrites. Most of them are myelinated, which is considered to be a characteristic of the axons. Nevertheless, they differ in the mechanisms for the membrane protein targeting. The axons of the

afferent neurons merge into the vestibulocochlear nerve, also known as the VIII. cranial nerve, relaying the information further to the central nervous system (Burack, 2000; Purves *et al.*, 2004).

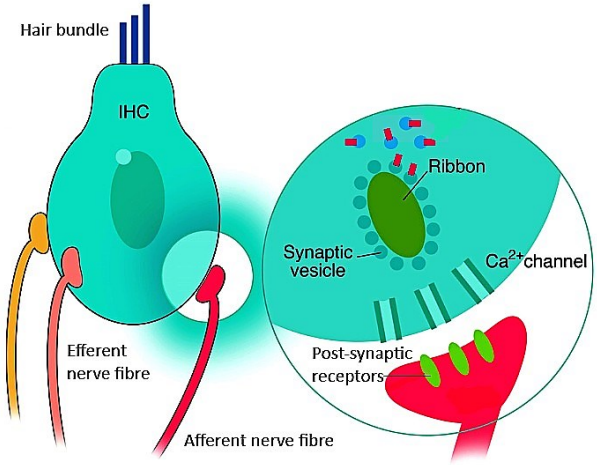

Figure 5. The afferent synapse. The ribbon synapse of inner hair cell (IHC) is characterized by the presence of synaptic bodies with an associated collection of synaptic vesicles inside the active zone of the presynaptic membrane. Adapted from (Sun *et al.*, 2018).

Inner ear hair cells and afferent neuronal processes form a uniquely specialized type of the synapse, known as the ribbon synapse. The structures called the synaptic bodies, or the ribbons distinguish the synapses. The ribbons can be found exclusively within sensory cells, while several variations between different types of sensory cells are evident. An example is syntaxin 3, whose presence was not recorded in inner ear hair cells, but is included in the ribbons of retinal cells (Von Kriegstein *et al.*, 1999; Safieddine and Wenthold, 1999). The structure of synaptic bodies reminds the composition of conventional synapses. However, the ribbons contain protein RIBEYE, with the components unique for the ribbon-kind of synapses (Schmitz, 2000). Synaptic bodies are located inside the active zone of the presynaptic membrane (Figure 5). A collection of small synaptic vesicles is attached to the ribbons by thin filaments. Inner hair cells contain a higher number of vesicles compared to outer hair cells (Weisz *et al.*, 2012). The synaptic vesicles include the neurotransmitters, allowing ongoing spontaneous and rapid neurotransmitter release (Safieddine and Wenthold, 1999). The depolarization of hair cells causes the influx of calcium ions at the active zones. Subsequently, neurotransmitters are released and stimulate the postsynaptic receptors on afferent neuronal processes, which carry the information to the brainstem (Beutner *et al.*, 2001).

3.2.1. The vestibular ganglion

The vestibular ganglion, called the Scarpa´s ganglion, is located in the lateral portion of the internal auditory meatus. Its shape resembles a distorted hourglass (Sato, 1992). It contains thousands of bipolar nerve cell bodies and provides the innervation of the sensory epithelium of the vestibular structures. Afferent processes of vestibular ganglion neurons capture incoming impulses. Their axons merge to create the vestibular nerve. The vestibular nerve alongside with the cochlear nerve form the vestibulocochlear nerve. Thereupon, the signal is transmitted further to the brain (Luo, 2016).

The vestibular ganglion consists of two portions divided by the narrow section called the *isthmus ganglionaris*. The superior division innervates the utricle, the superior semi-circular canal, and the lateral semi-circular canal, while the inferior region innervates the saccule and the posterior semi-circular canal (Khan and Chang, 2013). The volume of the vestibular ganglion seems to remain the same throughout life, but the aging causes a decrease in the density of vestibular hair cells as well as neurons innervating the area (Sato, 1992). Interestingly, specific vestibular neurons show spontaneous restoration of synaptic contacts and regeneration of neuronal processes. The possibility of restoring the damaged vestibular epithelium function symbolizes notable progress in the maintenance and treatment of the vestibular synapses (Travo, 2012).

3.2.2. The auditory ganglion

The auditory ganglion, called the spiral ganglion, is located in the modiolus within the cochlea. The ganglion consists of the somata of spiral ganglion neurons (SGNs). Their peripheral processes link with the organ of Corti, while the central processes merge into the cochlear nerve, responsible for delivering the acoustic information from cochlear hair cells to the brain. The spiral ganglion is made up of Type I and Type II sensory neurons (Figure 6) (Echteler, 1992).

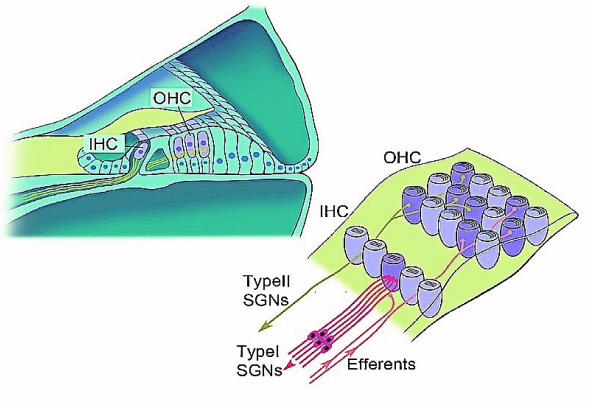

Figure 6. The spiral ganglion neurons. Type I SGNs innervate inner hair cells, while Type II SGNs connect outer hair cells and supporting cells within the cochlea. Adapted from (Sun *et al*., 2018).

Type I SGNs are large, bipolar, and myelinated. They make up approximately 95% of the spiral ganglion population. Individual SGNs extend unbranched peripheral processes toward inner hair cells, a total of 5-30 sensory neurons innervates each inner hair cell in the mature mammalian cochlea. Specialized innervation helps convey sensory information with high resolution. Type I SGNs can be further divided into three genetically distinct subtypes, known as Type Ia, Ib, and Ic SGNs. Type Ib represents most of the Type I SGN population, Type Ic remains a minority. The subclasses exhibit significant variations, such as differences in expression of transcription factors, neurotransmitter receptors, channel subunits, or cell adhesion molecules. Type Ia, Ib, and Ic SGNs show different spatial arrangements as well as high selectivity for a limited range of frequencies. Additionally, Type I SGNs exhibit differences in spontaneous firing rates. Thus, they can be classified as low-SR, medium-SR, and high-SR fibers. Whereas the total number of SGNs decreases with age, Type Ic SGNs seem to be particularly vulnerable

to noise or aging, indicating that the synapse damage is closely related to hearing loss (Petitpré *et al.*, 2018; Shrestha *et al.*, 2018; Sun *et al.*, 2018).

Type II SGNs make up the remaining 5% of the population of spiral ganglion neurons. These neurons are bipolar or pseudomonopolar and have small to average-size somata. Their peripheral processes remain unmyelinated and thin. The processes extend along the cochlear spiral while contacting multiple outer hair cells as well as supporting cells, such as Dieters' cells and Hensen's cells (Fechner *et al.*, 2001; Reid, 2004). Type II SGNs, although being a minority, show a fascinatingly wide range of functions. One of the features is the activation of medial olivocochlear efferents to suppress cochlear amplification. Besides, a gradual loss of Type II SGNs may play an important factor in age-related hearing loss (Froud *et al.*, 2015). Type II SGNs are also involved in auditory nociception. ATP release from damaged cells after loud noise exposure or mechanical trauma modulates the activation of afferents, showing the importance of Type II SGNs as pain-sensing agents within the auditory system. These abilities may help to protect the cochlea and avoid painful noises (Weisz, 2009). Additionally, Type II SGNs show slower kinetics and accommodation compared with Type I SGNs (Reid, 2004).

4. The auditory pathway

The auditory pathway ensures the transition of the sound frequency information from the cochlear region to the corresponding areas of the cortex, along with the processing and evaluation of received information. The pathway remains fast and relatively short, with only four relays (Figure 7). The first neurons of the auditory pathway are SGNs within the cochlea. Their peripheral processes receive the information from hair cells, while the central axons form the cochlear division of the vestibulocochlear nerve. Initially, the fibers of the cochlear nerve bifurcate to form two branches. The branches synapse with the neurons of the dorsal cochlear nucleus and the ventral cochlear nucleus in the brainstem. The nerve further subdivides the ventral cochlear nucleus into two sections, the anteroventral cochlear nucleus and the posteroventral cochlear nucleus. At this level, basic signals like intensity and frequency are decoded. Cochlear nuclei neurons represent the second neurons of the pathway. They

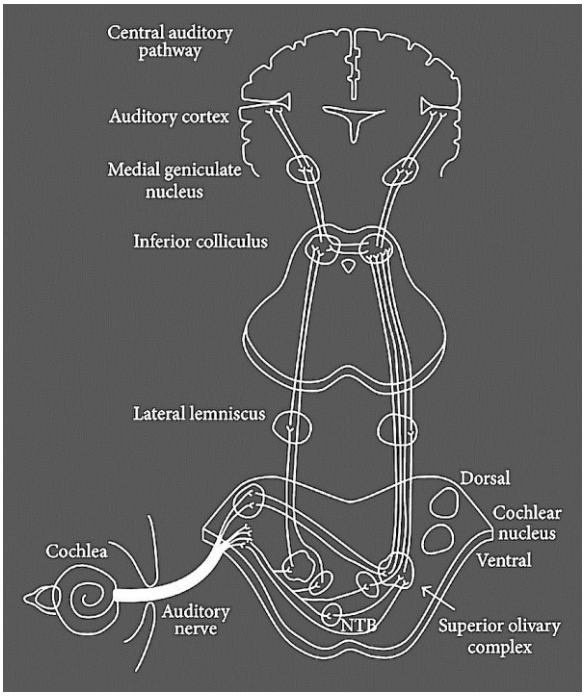

Figure 7. The auditory pathway. Diagram of the central auditory pathway from the inner ear to the auditory cortex. The pathway is relatively short and fast due to large myelinated fibers. The pathway includes several auditory nuclei. Adapted from (Lucker and Doman, 2015).

project to the inferior colliculus, which is the nucleus located in the midbrain. Within the inferior colliculus, the axons of cochlear nuclei neurons form synapses with the third neurons of the auditory pathway. Subsequently, the third neurons carry the message up to the level of the medial geniculate body in the thalamus. Neurons of the medial geniculate body proceed to the primary auditory cortex, where the information is recognized and evaluated. A section of axons from the cochlear nuclei passes through the series of additional auditory nuclei, such as the superior olivary complex, the trapezoid body, and the lateral lemniscus. These nuclei have a significant role in decoding and integration. A vital feature of the auditory pathway is also the decussation of numerous nerve fibers. Thus, the unilateral damage to the auditory pathway generally does not cause hearing loss (Cramer and Gabriele, 2014; Králiček, 2004; Luo, 2016).

The subdivision of the auditory pathway, called a non-lemniscal pathway, does not reach the primary auditory cortex. On the contrary, it projects more diffusively to the belt areas of the auditory cortex, while neuronal responses seem to be less specific (Parras *et al.*, 2017).

4.1. Tonotopic organization of auditory circuits

The fundamental principle of mammalian hearing system, known as the tonotopy, is precise spatial segregation of sounds based on frequency. Sensory hair cells are tonotopically organized according to sound frequencies along the cochlear spiral. Inner hair cells at the base respond to the highest frequencies, while those at the apex respond to the lowest frequencies. Hair cells transmit the information to SGNs. Because of the specialized innervation within the cochlea, auditory processes respond to a narrow range of frequencies, with the maximal response at one particular frequency. It is known as the "best frequency" for an individual neuron (Appler and Goodrich, 2011).

Furthermore, Type Ia, Ib, and Ic SGNs show different proportions along the tonotopic axis of the cochlea. Even though the proportions of SGN subtypes are similar at the apex and the middle turn, Type Ia SGNs form the majority at the cochlear base. Each SGN subtype is sensitive to a specific frequency corresponding to its position along the cochlear portion. Thus, Type Ia, Ib, and Ic SGNs are, much like inner hair cells, tonotopically organized with different sensitivity to sounds and spontaneous firing rates (Shrestha *et al.*, 2018; Sun *et al.*, 2018). The spatial segregation is maintained at all stages of the auditory pathway up to the cortex (Appler and Goodrich, 2011).

Although neurons projecting to the primary auditory cortex tend to be organized tonotopically, this principle is not evident in neurons of the non-lemniscal pathway (Parras *et al.*, 2017).

5. Development of the inner ear

The mammalian inner ear is a highly organized and complex structure containing a multitude of different cell types. Numerous transcription factors and signaling pathway regulatory control the development of the inner ear as well as the differentiation, maturation, and survival of spiral and vestibular neurons (Morsli *et al.*, 1998).

5.1. Gross development of mouse inner ear

The inner ear develops on either side of the hindbrain from the pre-placodal region, a molecularly diverse domain in the neural plate border. The pre-placodal region gives rise to placode precursors. The next step of inner ear development is the formation of the otic placode, consisting of transient thickening of ectoderm (Streit, 2004). During the neurulation at embryonic day 9 (E9), the otic placode invaginates and pinches off from surface ectoderm. At E9.5, a structure known as the otic vesicle or the otocyst is formed. The otocyst undergoes a series of morphological events, resulting in the mature inner ear. After the formation of the otocyst, the otic epithelium acquires its positional identity along the three primary axes responsible for the establishment of inner ear structures: the anterior-posterior axis, dorsal-ventral axis, and medial-lateral axis. Subsequently, individual neuroblasts delaminate to form the statoacoustic ganglion (SAG). The ganglion contains neural precursors of the spiral and vestibular ganglia and is responsible for innervation of the developing ear. By this stage, the neural, sensory, and non-sensory cell fates are established. At E12.5, the cochlear region acquires a more complex shape, and the future parts of the vestibular system are defined as small bulges. The underlying architecture of mouse inner ear is established at E14.5, though the development and maturation of the inner ear are completed after the birth (Figure 8) (Barald and Kelley, 2004; Morsli *et al.*, 1998; Wu, 1998).

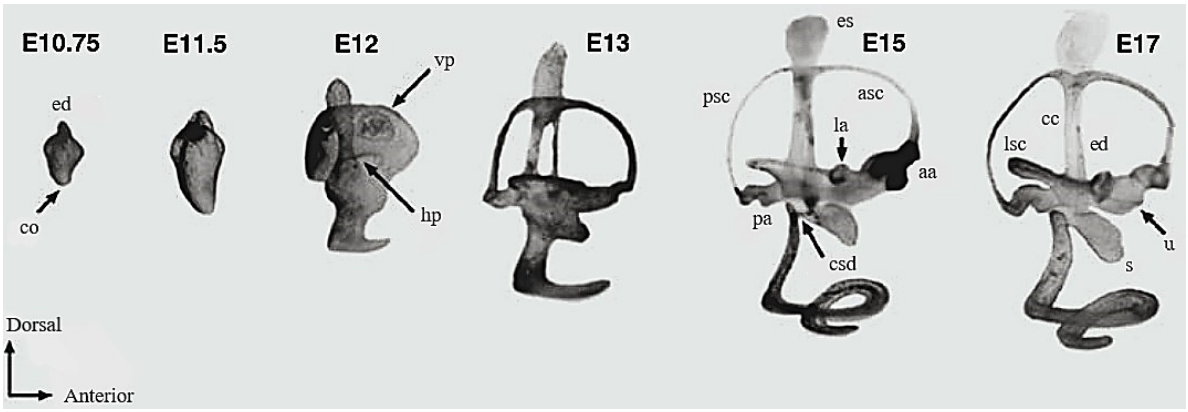

Figure 8. Morphological development of mouse inner ear. Lateral view of the right inner ear from E10.75 to E17. Abbreviations: aa, anterior ampulla; asc, anterior semi-circular canals; cc, common crus; co, cochlear duct; csd, cochlear saccular duct; ed, endolymphatic duct; es, endolymphatic sac; hp, horizontal canal pouch; la, lateral ampulla; lsc, lateral semi-circular canal; pa, posterior ampulla; psc, posterior semi-circular canal; s, saccule; u, utricle; vp, vertical canal pouch. Adapted from (Morsli *et al.*, 1998).

5.2. Specification of sensory epithelium

The functional unit of the mouse inner ear consists of neurons, mechanotransducing hair cells, and supporting cells. Different cell types are derived from a common otic progenitor located in the neurosensory competent domain of the otic vesicle. Proneural precursors give rise to neurons of the

inner ear, while sensory hair cells and supporting cells are derived from prosensory precursors. Transcription factors SOX2, ATOH1, and LHX3 appear to be indispensable for prosensory specification (Dabdoub *et al.*, 2008; Hume, 2007).

SOX2 is an HMG box transcription factor essential for the specification of prosensory domain. SOX2 is expressed through all development stages from E10.5. Its misexpression causes failure of prosensory domain establishment and formation of anomalous sensory epithelium with disorganized sensory hair cells. Mutations of SOX2 expression lead to hearing loss (Dabdoub *et al.*, 2008; Kiernan *et al.*, 2005). Although the presence of SOX2 is required for the specification of sensory epithelium, SOX2 acts as the negative regulator of hair cell formation, showing antagonistic functions during inner ear development. SOX2 initiates the expression of ATOH1, a bHLH transcription factor vital for the differentiation of sensory hair cells. Subsequently, the expression of ATOH1 leads to induced hair cell formation and down-regulation of SOX2, which remains present in differentiated supporting cells. ATOH1 expression is not detectable in the mature inner ear (Neves *et al.*, 2012).

Notch signaling controls the specification of sensory epithelia. The fundamental principle of Notch signaling is based on interactions between the transmembrane receptor NOTCH1 in one cell and Notch ligands JAG2 and DLL1 in neighboring cells. JAG2 and DLL1, present in nascent sensory hair cells, act synergistically. Both are required for hair cell formation. This pathway has a dual role in inner ear development, using mechanisms of lateral induction and lateral inhibition. The process of lateral induction is necessary for sensory specification. Inner ear cells expressing Notch ligands stimulate neighboring cells to turn up Notch activation in order to differentiate from the initial population (Eddison, 2000). Lateral inhibition, on the other hand, regulates pattern formation of the developing inner ear. JAG2 and DLL1 signal through the NOTCH1 receptor, preventing adjacent cells from differentiating as sensory hair cells and thus creating a mosaic of diverse cell types (Kiernan *et al.*, 2005; Petrovic *et al.*, 2014).

LHX3 is a LIM homeodomain transcription factor involved in the specification of sensory hair cells. During development stages, LHX3 is expressed in auditory and vestibular sensory epithelium. In mature inner ear, however, its expression persists exclusively within vestibular hair cells (Hume, 2007).

5.3. Specification of inner ear neurons

The otic placode becomes subdivided into non-neurogenic and neurogenic domains early in inner ear development. The proneural domain gives rise to spiral and vestibular neurons. At E9.5, neuroblasts delaminate from the otic vesicle and migrate to the side of the nascent ganglion. Subsequently, otic neuroblasts differentiate to form primary neurons of the cochlear and vestibular ganglia, connecting the inner ear to the central nervous system. As nascent neurons mature and specify, various groups of transcription factors affect their differentiation, final location, and survival at different development stages (Dabdoub, 2016; Morsli *et al.*, 1998).

6. Molecular mechanisms of inner ear neurogenesis

Both expression and inhibition of SOX2 are crucial for proper development of mouse inner ear (Evsen *et al.*, 2013; Kiernan *et al.*, 2005). Key factors of neuronal differentiation, maturation, and survival include bHLH genes *Neurog1* and *Neurod1* (Liu *et al.*, 2000; Ma *et al.*, 1998).

Additional signaling pathways and transcription factors involved in the development of otic neurons include ISL1, POU4F1, and TBX1 (Dykes *et al.*, 2011; Sun *et al.*, 2008; Vitelli *et al.*, 2003). Cochlear and vestibular neuronal identity is closely linked to the expression of zinc finger transcription factor GATA3, essential for the coordinated development of auditory neurons (Lawoko-Kerali *et al.*, 2004). Neurotrophins, BDNF and NTF3, and associated receptors are critical for survival of vestibular and spiral ganglia (Agerman *et al.*, 2003; Fritzsch *et al.*, 1997).

6.1. Expression of SOX2

SOX2 transcription factor is involved in the specification of neurons, sensory hair cells, and supporting cells in the mammalian inner ear. SOX2 is initially expressed during early development stages of the neurosensory domain in the otic vesicle. Its expression is important for proliferating neuronal lineage cells. SOX2 up-regulates bHLH proneural genes *Neurog1* and *Neurod1*, responsible for the differentiation of sensory neurons (Graham *et al.*, 2003; Neves *et al.*, 2007).

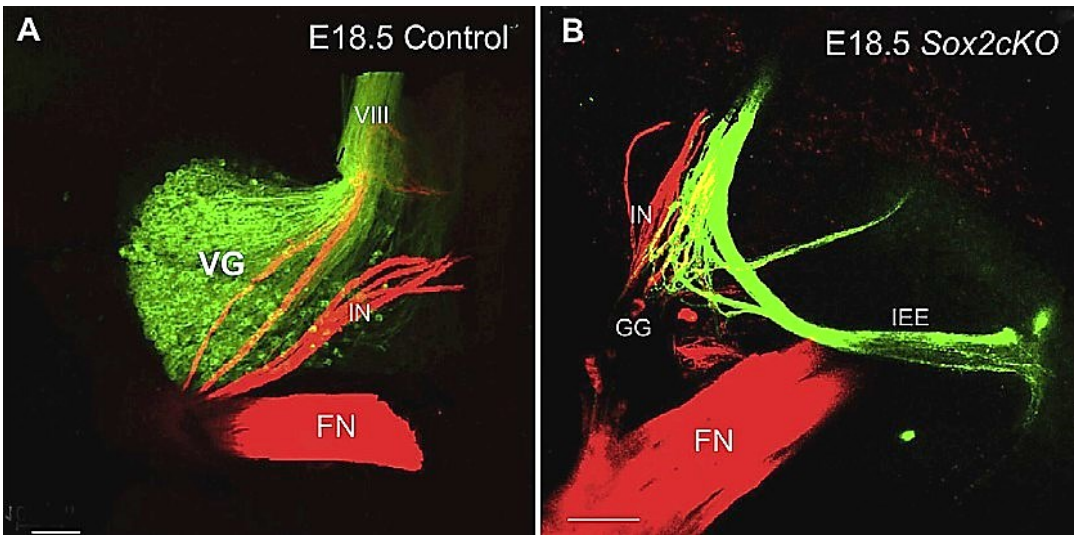

Figure 9. Absence of afferents in the *Sox2*cKO inner ear. Vestibular (VG) neurons and afferent fibers forming the vestibulocochlear nerve (VIII. cranial nerve) are visible in the E18.5 control mouse (**A**). In *Sox2*cKO mutant, no afferent fibers and no vestibular ganglion neurons are detected. Efferent fibers (IEE) of the mutant are reduced and disorganized (**B**). Abbreviations: VIII, vestibulocochlear nerve; FN, facial nerve; GG, geniculate ganglion; IN, intermediate nerve. Adapted from (Dvorakova *et al*., 2020).

The progression of otic neurogenesis is associated with down-regulation of SOX2 expression. Both NEUROG1 and NEUROD1 inhibit SOX2 expression, thus preventing the extension of sensory epithelia and allowing differentiation and specification of nascent neurons (Evsen *et al.*, 2013).

Although SOX2 appears to be crucial for proper inner ear development (Steevens *et al.*, 2017), recent studies suggest that SOX2 is not necessary for early otic neurogenesis (Dvorakova *et al*., 2020), as previously believed. The otic placode invagination and NEUROG1 expression can proceed without the presence of SOX2. At the same time, however, SOX2 expression remains critical for later stages of inner ear development (Figure 9) (Dvorakova *et al.*, 2020). The formation of sensory epithelia fully depends on continuous expression of SOX2. At later stages of neurosensory development, neurotrophins BDNF and NTF3 are released from sensory epithelium. The survival of nascent neurons is critically dependent on these proteins. Without well-developed sensory epithelia and proper neurotrophic support, early formed neurons eventually die by apoptosis (Dvorakova *et al.*, 2016; Dvorakova *et al.*, 2020).

6.2. Regulation of NEUROG1

The bHLH transcription factor NEUROG1 drives neuronal development in the inner ear. At E9.5, *Neurog1* is initially expressed in the neurosensory domain of the otic vesicle prior to the delamination of neuroblasts from otic epithelium. NEUROG1 is essential for proneural fate decision making. The inability of neuroblast delamination and complete absence of inner ear neurons in the *Neurog1* null mouse demonstrate the importance of NEUROG1 for neuronal development (Figure 10) (Koundakjian, 2007; Matei *et al.*, 2005). Soon after the delamination of nascent neurons, the expression of *Neurog1* is down-regulated. NEUROG1 precedes and induces the expression of target gene *Neurod1*, crucial for further neuronal differentiation (Andermann, 2002; Ma *et al.*, 1998).

The functions of NEUROG1 and ATOH1 show mutual antagonism. *Neurog1* is expressed in precursors of ganglion neurons before the expression of genes required for prosensory differentiation. Although the activity of *Atoh1* is noticeable early in development, the protein levels of ATOH1 are reduced during otic neurogenesis and remain undetected until later development stages. For that reason, inner ear neurons develop in advance of sensory hair cells. NEUROG1 is a primary candidate for mediating the repression of ATOH1 by protein-protein interactions and thus preventing the formation of sensory epithelia (Gálvez *et al.*, 2017; Raft *et al.*, 2007).

Until recently, it was assumed that the expression of NEUROG1 is regulated by SOX2 (Ma *et al.*, 1998). *Sox2* expression is subsequently inhibited at transcriptional level by NEUROG1, suggesting that down-regulation of SOX2 is a key component of inner ear neurogenesis (Evsen *et al.*, 2013). However, SOX2 protein is not necessary for early inner ear development (Dvorakova *et al.*, 2020) (for details, see chapter 6.1). Further studies indicate that initial expression of *Neurog1* is activated by a combination of several transcription factors, including SIX1 and EYA1. Both factors function upstream of *Neurog1*, taking part in the induction of neuronal fate and regulation of the differentiation of the mouse inner ear. The importance of SIX1 and EYA1 is proven by a complete failure of neuronal development in the

double deletion *Six1-Eya1* mutant. SOX2, if present, works cooperatively with SIX1 and EYA1 in order to select a portion of ectodermal cells adopting neuronal fate by inducing *Neurog1* expression. Subsequently, SIX1 and EYA1 seem to interact with *Neurog1* to induce the expression of *Neurod1*. SIX1 and EYA1 are likely to have a role in later stages of inner ear development while regulating additional downstream bHLH factors (Ahmed, 2012; Zheng *et al.*, 2003).

The absence of *Neurog1* affects the formation of sensory hair cells. Although sensory hair cells of *Neurog1* null mutant show no structural abnormalities, the inner ear of the mutant exhibits smaller-size sensory epithelia. It appears that a certain number of progenitor cells, depending on the expression of *Neurog1*, ultimately forms not only otic neurons but also sensory hair cells and supporting cells. This evidence suggests the existence of a population of common neurosensory progenitors that can differentiate in both sensory and neuronal cells. In the *Neurog1* mutant, these progenitors eventually die, which explains the reduction of length and width of inner ear sensory epithelia (Ma, 2000).

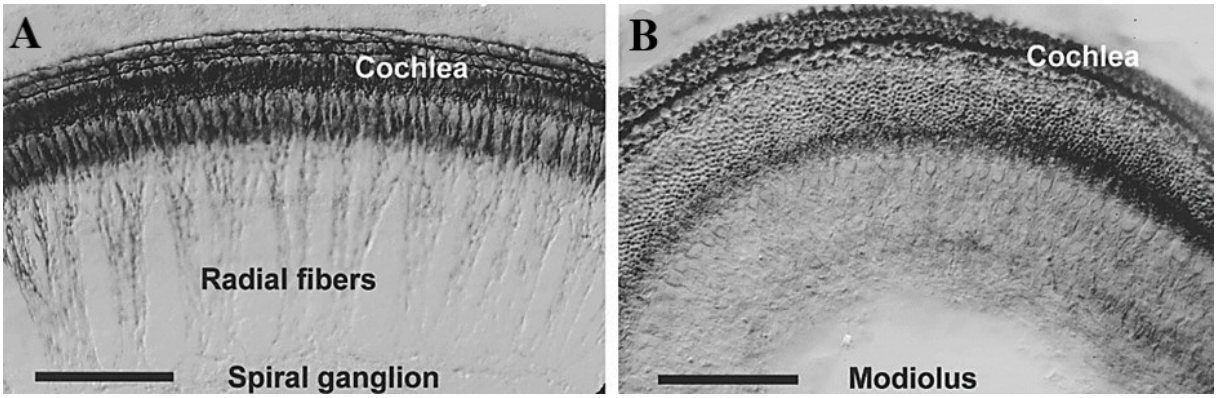

Figure 10. Absence of cochlear nerve fibers in the *Neurog1*cKO mouse. Spiral sensory afferent and efferent fibers are visible as radial fibers in the cochlear modiolus of a P0 control mouse (**A**). *Neurog1* null mutant has smaller-size cochlea with no afferent or efferent fibers. (**B**). Adapted from (Ma *et al*., 2000).

New publications provide compelling solutions and research directions. NEUROG1 appears to replace the function of ATOH1 partially during cochlear development. Although *Neurog1* cannot fully rescue sensory hair cell differentiation, it can ensure functional support for the limited survival of developed sensory epithelia (Jahan *et al.*, 2015). As part of the research, the overexpression of *Neurog1* within the mouse inner ear was used to differentiate an immortalized cell line of multipotent otic progenitors into auditory neurons. Generated cochlear neurons may be able to reverse hearing loss. However, various stem cell lineages express NEUROG1 in order to make different types of neurons. The converting process may lead to increased cell proliferation and thus poses a possible cancer risk. Additionally, the study showed that the expression of NEUROG1 influences chromatin status. Targeted changes in chromatin state before the overexpression of NEUROG1 could prevent unwanted cell

proliferation (Song *et al.*, 2017). According to recent findings, a combination of NEUROG1, NEUROD1, and other transcription factors may be able to reprogram cochlear neonatal glia cells into SGNs *in vivo*. Cochlear neurons do not show regenerative capacity. Therefore, the possibility of *in situ* regeneration would be a significant step forward in the treatment of permanent hearing impartment caused by degeneration of inner ear neurons (Li *et al.*, 2020).

6.3. The role of NEUROD1

The bHLH transcription factor NEUROD1 is vital for the differentiation, maturation, and survival of inner ear neurons. It is downstream target of NEUROG1 (Ma *et al.*, 1998).

NEUROD1 cross-regulates transcription factors required for the specification of inner ear cells. The expression of NEUROD1 suppresses the prosensory gene *Atoh1*, crucial for sensory hair cell formation. The conditional knockout of *Neurod1* causes premature expression of multiple genes determining cell specification, resulting in ectopic hair cell formation and disorganized sensory epithelium within the cochlea (Jahan *et al.*, 2010b).

The conditional deletion of *Neurod1* leads to the significant reduction of inner ear neurons (Macova *et al*., 2019). Unlike the *Neurog1* null mouse displaying a complete loss of all neurons, some of the neurons survive in the *Neurod1* mutant. These are mostly vestibular neurons. However, the volume of both spiral and vestibular ganglia is significantly reduced. The spiral ganglion is nearly absent by E14.5, as opposed to thousands of auditory fibers in a control mouse. The remaining neurons,

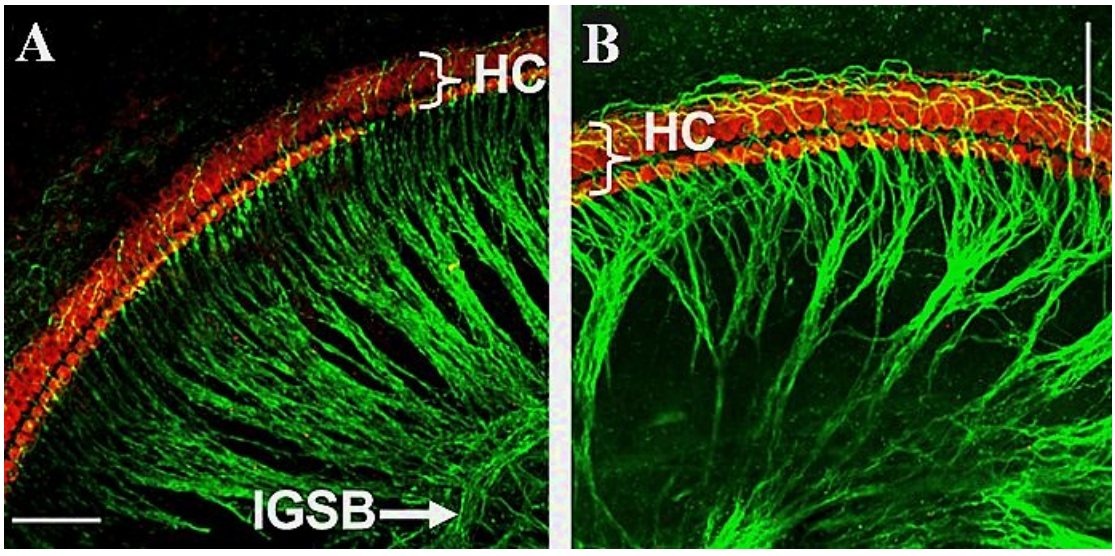

Figure 11. Disorganization of cochlear fibers in the *Neurod1*cKO mouse. Arranged radial fibers (labeled by anti-tubulin, green) innervate sensory hair cells (HC), which are labeled by anti-Myo7a (red) at the apex of a P0 control mouse (**A**). In contrast, neuronal fibers of P0 *Neurod1*cKO mice show significant changes in the number and spatial organization. No intraganglionic spiral bundle (IGSB) is visible in *Neurod1*cKO mouse (**B**). Adapted from (Macova *et al*., 2019).

disorientated and abnormally branched, are located mostly in the middle turn of the cochlea, while the fibers seem to innervate as many sensory hair cells as possible (Figure 11). Few neurons are noticeable at the apex and the base. The expression of additional bHLH genes appears to ensure the survival of remaining auditory neurons. (Kim *et al.*, 2001; Krüger *et al.*, 2006; Liu *et al.*, 2000).

The absence of *Neurod1* affects tonotopic organization of the cochlea, resulting in disorganized primary tonotopy map and altered features of mouse auditory system (Macova *et al.*, 2019). Inner ear nerve fibers form a single root in the *Neurod1* mutant, projecting to both vestibular and auditory nuclei. The somata of vestibular neurons are located in the internal auditory meatus, while cell bodies of auditory neurons are in the modiolus. Peripheral fibers of surviving neurons fail to segregate and form two branches of the innervation in the cochlear nuclei in the brainstem. Thus, NEUROD1 has a particularly profound role in the regulation of neuronal branching, organization of projecting afferent

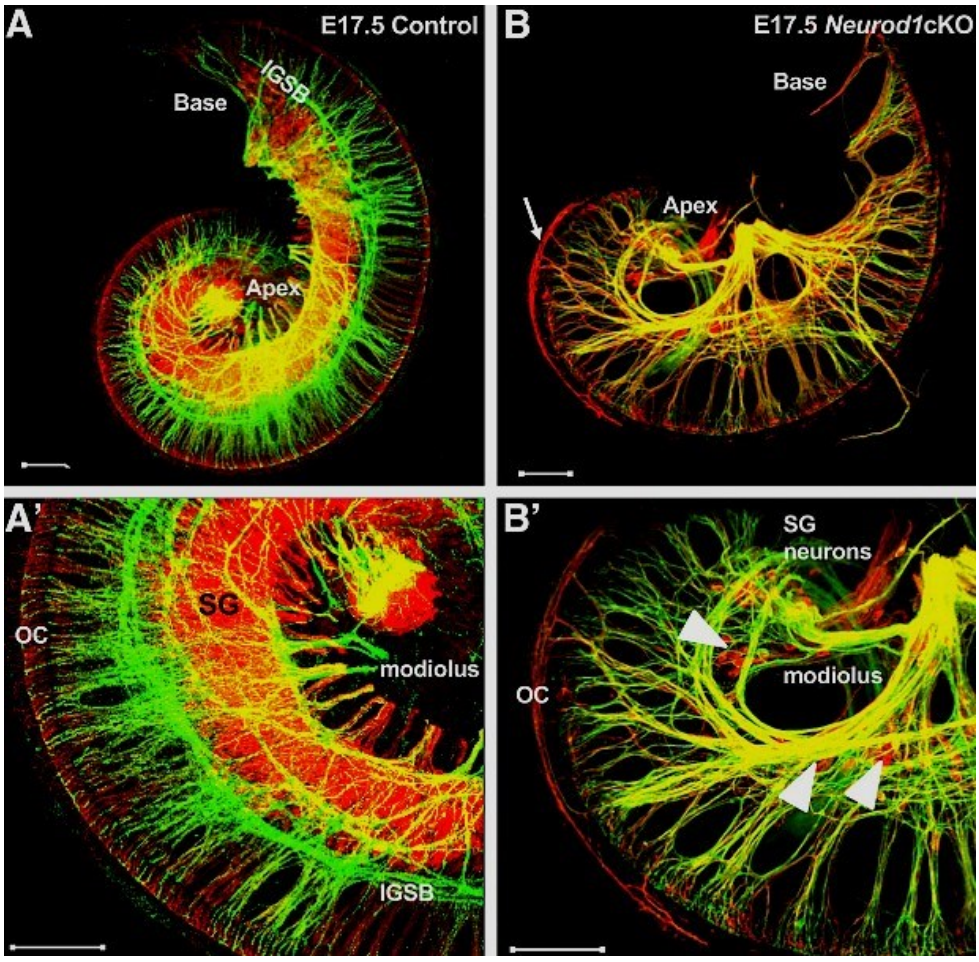

Figure 12. Disorganized efferent fibers in the *Neurod1*cKO mouse. In E17.5 control mouse, organized efferent fibers (lipophilic green dye) form the intraganglionic spiral bundle (IGSB) along spiral ganglion neurons (SG; lipophilic red dye) (**A**), and afferent fibers reaching the organ of Corti (OC; lipophilic red dye) (**A´**). Efferent fibers of *Neurod1*cKO mouse lack the formation of IGSB, though the fibers manage to reach outer hair cells (**B**). Afferent innervation is significantly reduced in a mutant (**B´**). Adapted from (Macova *et al*., 2019).

and efferent fibers, tonotopic organization of the auditory system (Macova *et al*., 2019) (Figure 12), and survival of differentiating otic neurons (Jahan *et al.*, 2010a; Kim *et al.*, 2001; Liu *et al.*, 2000).

Several studies indicate that non-neural cells of the auditory ganglion could be reprogrammed into induced neurons *in vivo* through ectopic expression of NEUROD1 and ASCL1 (Nishimura *et al.*, 2014). A promising candidate is a cluster of spiral ganglion glial cells within the Rosenthal´s canal. Further research is needed to determine whether induced neurons form connections with the fibers of the auditory circuits. Possible production of induced neurons *in vivo* may help with the regeneration of auditory neurons and potential treatment of hearing loss (Nishimura *et al.*, 2014; Noda *et al.*, 2018).

6.4. Additional transcription factors involved in inner ear neurogenesis

Besides the bHLH genes *Neurog1* and *Neurod1* involved in the development of inner ear neurons, multiple additional transcription factors affect otic neurogenesis.

GATA family gene *Gata3* has a crucial role in cell recognition and pathfinding during inner ear development. GATA3 is initially expressed in all sensory epithelia of the otic placode. Its expression decreases relatively early in vestibular neuroblasts. By E14.5, GATA3 is not detectable in vestibular sensory epithelia. However, the expression of GATA3 remains strong in delaminating auditory neuroblasts, taking part in the coordination of timing and specification of these neurons (Lawoko-Kerali, 2002; Rivolta and Holley, 1998). GATA3 is required for activation of NEUROD1 in cochlear neurons, regulating expression patters of NEUROD1, and differentiation of SGNs. Thus, two neuronal populations appear to be specified before their delamination from the otic vesicle (Lawoko-Kerali *et al.*, 2004). Continuous expression of GATA3 through developmental stages is required for proper neurosensory differentiation within the cochlea. The expression of GATA3 continues through the late postnatal stages. Conditional deletion of *Gata3* causes a reduction of prosensory cells and unusual hair cell formation. It seems that GATA3 enhances *Atoh1* activity and enables sensory hair cell differentiation. The absence of *Gata3* leads to disruption of cochlear wiring, the formation of disorganized peripheral projections, and incorrect patterning within the cochlea. At P0, a massive loss of auditory neurons is recorded in the *Gata3*cKO mouse (Figure 13). Besides, GATA3 ensures the repression of vestibular genes in SGNs, which are crucial for cell fate determination. The haploinsufficiency of *Gata3* leads to degeneration of the auditory system and deafness. Thus, GATA3 remains vital for coordinated development of the auditory system, regulation of cochlear wiring, and survival of SGNs (Appler *et al.*, 2013; Duncan and Fritzsch, 2013; Van Der Wees *et al.*, 2004).

The homeodomain transcription factors POU4F1 and ISL1 are synergistically required for cell differentiation and regulation of gene expression during inner ear neurogenesis. At E9.5, both factors are expressed in developing projection neurons within the inner ear, although the presence of ISL1 is noticeable a little earlier, suggesting *Isl1* may act upstream of *Pou4f1* (Deng *et al.*, 2014). Targeted deletion of *Pou4f1* results in a smaller-size spiral and vestibular ganglia, several defects in innervation, and suppression of multiple genes, including neurotrophins receptor gene *Ntrk3* (Huang *et al.*, 2001).

During early development stages, *Pou4f1* is expressed in all types of inner ear neurons. Beginning at E16.5, however, *Pou4f1* is down-regulated. By P0, only 30% of auditory neurons maintain expression of POU4F1 in adulthood. These neurons appear to be a single subpopulation of Type I SGNs, most likely a subgroup of low-SR fibers (Sherrill *et al.*, 2019). Conditional knockout of *Isl1* causes significant deficits in peripheral innervation and reduced levels of *Ntrk1* and *Ntrk2* gene expression (Dykes *et al.*, 2011). *Pou4f1/Isl1* double mutants show defects in the axonal projections, neuronal innervation, and subtype specification. Although POU4F1 and ISL1 do not regulate expression of one another, current data indicate there is a strong interaction between two genes. Their co-expression regulates a cascade of multiple genes required for proper inner ear development. POU4F1 and ISL1 are required regulators and repressors of various bHLH genes, including *Neurod* class genes. The deletion of the factors leads to abnormal and persisted expression of bHLH genes at E13.5. POU4F1 and ISL1 have an essential role in the differentiation, innervation, and survival of inner ear neurons (Dykes *et al.*, 2011; Sun *et al.*, 2008)

T-box transcription factor TBX1 is involved in inner ear neurogenesis, acting as a negative regulator of neuronal development. Gain-of-function of the *Tbx1* gene displaces the expression of *Neurod1* and suppresses posteroventral otic neurogenesis. Conversely, *Tbx1* loss-of-function causes ectopic posterolateral expression of *Neurod1* and an increased number of neurons located in a portion associated with sensory epithelium by E9.5. *Tbx1* is a candidate gene for a 22q11.2 deletion syndrome (22q11.2DS), causing cardiac, parathyroid, and thymus defects. Absence of *Tbx1* results in aplasia of

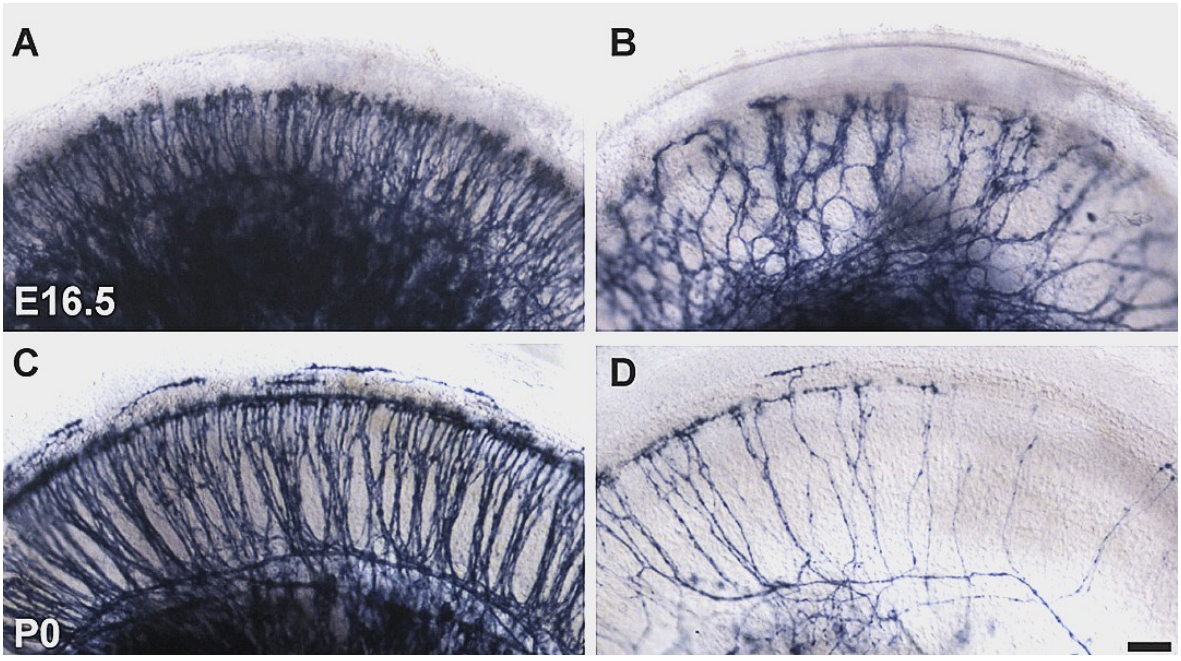

Figure 13. Reduced number of SGNs in the *Gata3*cKO mouse. By E16.5, SGN projections are visibly disorganized and decreased in number in the *Gata3*cKO mouse (**B**), compared with controls (**A**). In P0 mutants, the loss of neurons is even more prominent (**D**), in contrast to the control mouse (**C**). Adapted from (Appler *et al*., 2013).

sensory epithelia within the mouse inner ear. Thus, TBX1 has a fundamental role in early otic morphogenesis and is required for the specification and regulation of cell identity within the otic vesicle (Arnold *et al.*, 2006; Raft *et al.*, 2004; Vitelli *et al.*, 2003).

Neurotrophins produced by inner ear sensory epithelia, BDNF and NTF3, are essential for development and survival of otic neurons. BDNF and NTF3 work together with tyrosine kinase receptors *Ntrk2* and *Ntrk3* expressed in inner ear neurons. While the disruption of *Ntf3* or its receptor *Ntrk3* causes a massive loss of auditory neurons, mutation in *Bdnf3* or *Ntrk2* significantly reduces the number of vestibular neurons. Within the auditory system, NTF3 appears to replace the function of BDNF and ensure partial innervation (Agerman *et al.*, 2003). Such a replacement was not observed within the vestibular system. The deletion of both neurotrophins and associated receptors leads to a complete lack of afferent innervation of the mouse inner ear (Fritzsch *et al.*, 1997; Agerman *et al.*, 2003). Neurotrophins BDNF and NTF3 seem to improve the survival of SGNs and stimulate regrowth of their peripheral fibers. Temporal neurotrophin therapy could improve the function of existing cochlear implants, which is a promising strategy for the treatment of hearing loss due to defects in the cochlear peripheral system (Budenz, 2012; Ramekers *et al.*, 2015).

7. Conclusion

During the early stages of inner ear development, the otic placode is divided into the non-neurogenic and neurogenic domain. Subsequently, the neurogenic domain gives rise to spiral and vestibular neurons. Nascent neurons are affected by numerous interconnected signaling pathways and transcription factors. The development of sensory epithelia and inner ear neurons cannot proceed without the presence of transcription factor SOX2. As otic neurogenesis continues, the expression of SOX2 is down-regulated in differentiating neurons.

Critical factors for the development and survival of inner ear neurons are bHLH genes *Neurog1* and *Neurod1*. NEUROG1 induces the expression of NEUROD1 and inhibits the expression of prosensory transcription factor ATOH1. In the absence of *Atoh1*, NEUROG1 partially replaces its function. The conditional deletion of *Neurog1* leads to a complete loss of neurons and smaller-size sensory epithelia. *Neurod1* null mouse shows a visible reduction of inner ear neurons. Few remaining neurons are disorganized, and the tonotopic organization of the cochlea is disturbed.

Transcription factor GATA3 affects the specification of spiral and vestibular neurons, organization of peripheral projections, and proper development of the auditory system. The absence of GATA3 disturbs cochlear patterning and leads to complete degeneration of auditory neurons. The homeodomain transcription factors POU4F1 and ISL1 are essential for proper development, specification, and innervation of inner ear neurons. Targeted deletion of POU4F1 and ISL1 leads to severe defects in afferent innervation, abnormal expression of bHLH genes *Neurog1* and *Neurod1*, and suppression of neurotrophin receptors *Ntrk1*, *Ntrk2*, and *Ntrk3*. T-box transcription factor TBX1 has a significant role in early otic neurogenesis. TBX1 serves as a negative regulator of neuronal development, while the absence of *Tbx1* results in the incorrect organization of cell identity within the inner ear. Neurotrophins BDNF, NTF3, and their receptors are required for proper differentiation and survival of otic neurons. *Bdnf/Ntf3* double mutants display a complete loss of inner ear innervation.

Understanding individual transcription factors and signal pathways that regulate the development of the inner ear is a crucial step in the treatment of hearing impairment and deafness. Up-to-date publications provide breathtaking results and expand our knowledge of neuronal development. It appears that several transcription factors, regulating differentiation of the auditory system, are replaceable. Thus, their absence may not affect the development and survival of cochlear neurons. The focus of the latest research is on regeneration and restoration of neuronal function. The possibility of reprogramming non-neural cochlear cells into functioning neurons through transcription factors such as NEUROG1 and NEUROD1 seems promising. These two factors may be a key to the regeneration of auditory neurons and future treatments of hearing loss. Therefore, NEUROG1 and NEUROD1 are ideal candidates for further research.

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